

PHEROMONE HYDROLYSIS BY CUTICULAR AND
INTERIOR ESTERASES OF THE ANTENNAE,
LEGS, AND WINGS OF THE CABBAGE LOOPER
MOTH, *Trichoplusia ni* (HÜBNER)¹

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Abstract—Examination was made of the hydrolytic activities of esterases obtained from the antennae, legs, and wings of 3-day-old cabbage looper moths, *Trichoplusia ni* (Hübner), by elution and by homogenation of those appendages. Pheromone hydrolysis in 1-min assays was monitored by use of tritium-labeled (Z)-7-dodecen-1-ol acetate and thin-layer chromatography to separate the reaction products. Listed according to the activities of the esterases obtained by homogenation, the organs were antennae > legs > wings. In contrast, the order according to the activities of the eluted esterases was wings > legs > antennae. Also, the eluted enzymes were less active than the esterases obtained by homogenization. The relatively high pheromone-hydrolyzing activity present in homogenized antennae suggests that the esterases originated inside the antennae and lends support to the hypothesis proposed in earlier investigations that pheromone-inactivating enzymes may play an important role in the olfactory process, possibly by clearing pheromone from the vicinity of the olfactory receptors. The esterases detected on the cuticle, on the other hand, may function by preventing surface accumulation of pheromone. The higher measured esterase activity in homogenates of prothoracic legs than of mesothoracic or metathoracic legs suggests that the prothoracic legs, which are used to clean the antennae of debris, may function by removing and degrading pheromone from the surface of antennae.

Key Words—Antennae, esterases, pheromone degradation, cuticle, olfaction, cabbage looper, *Trichoplusia ni*, Lepidoptera, Noctuidae.

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dried and transferred to a silica gel SEP-PAK®. The SEP-PAK was eluted with 2 ml *n*-hexane (eluate was discarded), then with 5 ml benzene containing 1% ethyl acetate (v/v). Radioactivity in the benzene eluate was measured by scintillation counting and was found to contain 21.0 mCi of labeled pheromone (70.5% recovery). The labeled pheromone was then shown to be $\geq 99\%$ pure by gas chromatography (1.8 m \times 6 mm Dexsil® 300 on 100–200 mesh Supelcoport®).

Insect Ringer's solution was prepared according to Ephrussi and Beadle (1936).

Elution of Esterases. Ringer's solution was used to elute esterases from the antennae, legs, and wings of 3-day-old adult cabbage looper moths, and elution time was 12 hr at 22°C. The antennae of each live adult were held in a capillary tube containing $\approx 60 \mu\text{l}$ of the solution. For each sex, the antennal eluates from 120 insects were combined, dialyzed against Ringer's solution, and concentrated with 500 μl with a ProDi Mem® (10,000 mol wt cut-off membrane) apparatus. The prothoracic, mesothoracic, and metathoracic legs on one side of the body of each insect were held in 100 μl of solution, and the wings of each insect were held in 100 μl of solution. For each sex, the combined eluants from the legs of 51 insects and from the wings of 102 insects were centrifuged at 20,000 *g* for 30 min, dialyzed, and concentrated to 500 μl as described above.

Homogenization of Tissues. Male and female antennae (80 pairs per sex), legs (12 pair each of prothoracic, mesothoracic, and metathoracic (per sex), and wings (12 pairs) were dissected from 3-day-old adult moths and homogenized in a solution of 0.5 M sucrose and 0.05 M Tris HCl (pH 7.5). The homogenates were sonicated for 30 sec (Biosonic III, minimum intensity) and centrifuged at 20,000 *g* for 30 min at 4°C; then the supernatants were dialyzed against Ringer's solution and concentrated by the procedure described above.

For one experiment, insects were examined as to the relative pheromone-hydrolyzing activities in their mesothoracic and metathoracic legs. Thus, legs of each type were excised and homogenized separately. Enzyme concentrates were prepared from the homogenates as described above and were assayed for protein content and pheromone-hydrolyzing activity.

Protein Assay. The supernatants were assayed for protein by the Bio-Rad® procedure (Anonymous, 1979), with bovine gamma-globulin as standard.

Pheromone Assay. The assay for hydrolysis of radiolabeled pheromone was as previously described (Ferkovich et al., 1980). Briefly, preliminary tests were conducted to determine the conditions for a linear rate of hydrolysis in the assay mixture. In these tests, conducted at 22°C, the amount of protein (enzyme) was kept between 5 and 15 μg , but the concentration of pheromone was varied from 2.0×10^{-6} to 1.5×10^{-5} M, and reaction time, from 1 to 5 min. Then, specific activity was determined under linear-rate conditions on the basis of four replicate 1-min assays.

INTRODUCTION

During the past several years many insect pheromones have been identified for practical use in insect control. However, as pointed out by Roelofs (1980), the successful use of pheromones in insect control also requires "... in depth physiological and behavioral studies of insects in conjunction with the chemical analyses." One aspect of insect physiology that has received little attention but that may play an important role in the insect's perception of and behavioral response to pheromones is enzymes that degrade the pheromone. Schneider (1970) first proposed that such enzymes may serve to clear the pheromone from the surface and/or interior of the antennae after transduction and also to clear the body surface of pheromone, which might otherwise desorb and interfere with communication of the insect.

In the cabbage looper, *Trichoplusia ni* (Hübner), pheromone-hydrolyzing esterases that can be isolated from the chemosensory hairs on the antennae appear to originate in the inner structure of the antennae, possibly as membrane-bound esterases (Ferkovich et al., 1980). These enzymes have a considerably higher activity per unit of protein than esterases isolated from tissues which lack chemosensory sensilla.

That pheromone-catabolizing enzymes are also localized on the cuticle of the insect has been deduced from in vivo studies with the silk moth, *Bombyx mori* (L.) (Kasang and Kaissling, 1972), and the cabbage looper, *T. ni* (Mayer, 1975). In these studies, the insect was first exposed to the pheromone; then, the antennae and legs were extracted with various solvents to yield the pheromone and its breakdown products. It was assumed that the apolar solvents, such as pentane, extracted only the nonpolar compounds in the outer layers of the cuticle, and that the polar solvent extracted pheromone and metabolites from the inner layers.

If indeed pheromone-degrading enzymes are present on the surface of the cuticle, the following questions arise: can these enzymes be isolated in an active form? And, if so, are their activities comparable to those of esterases found inside the antennae? We now report a study to compare the activities of esterases eluted from the antennae and other appendages with those of esterases obtained by homogenization of those appendages.

METHODS AND MATERIALS

Chemicals. Tritiated (*Z*)-7-dodecen-1-ol acetate (200 mCi/mmol) was prepared by treating a benzene solution of (*Z*)-7-dodecen-1-ol (0.149 mmol) with an approximately two-fold molar excess of [³H]acetic anhydride (400 mCi/mmol; New England Nuclear) in the presence of a little pyridine. After standing at room temperature 2 days, the mixture was partitioned between cyclohexane and 7% aqueous sodium bicarbonate. The organic phase was

RESULTS AND DISCUSSION

The esterases obtained by homogenization of male and female antennae and legs (Figure 1A,B) had a higher specific activity than the corresponding eluted enzymes (Figure 1C,D). The activity of the esterases isolated from the wings by homogenization was similar to that obtained by elution.

During 1 min of assay, the actual amounts of product formed per microgram of antennal protein obtained from males to females, respectively, were 6.3 and 4.9 pmol for the homogenization-derived enzymes, and 0.2 and 0.1 pmol for the eluted enzymes. Ferkovich et al. (1980) reported that the esterases they isolated from chemosensory sensilla by fracturing the tips of the hairs on *T. ni* antennae had been weakly membrane bound (extrinsic membrane proteins) and could be released from the hairs by homogenization. In our study the antennal esterases isolated by homogenization were more active in hydrolyzing the pheromone than those isolated by elution. Thus, the esterases within the antenna are probably involved in degrading the pheromone and/or other nonpheromone chemicals at or near the membrane receptor sites. On the other hand, the esterases on the surface of the antennae probably facilitate pheromone deposition because of their low activity. Otherwise, the pheromone would be rapidly degraded on the antennal surface, and the probability of pheromone molecules reaching nerve-membrane receptor sites within the antenna would be lower. The higher cuticular esterase activity on legs and wings than on antennae suggests that esterases serve to keep the cuticle of legs and wings free of pheromone. This suggestion becomes more meaningful in light of the high amount of pheromone released per female. One female cabbage looper moth releases an average of 812 ng of pheromone per evening (Bjostad et al., 1980). Although adsorption of released pheromone onto the epicuticle of insects within the vicinity of calling females has not been shown, it is tempting to speculate that such adsorption does occur and is followed by hydrolysis of the pheromone by the cuticular esterases reported in this paper. If the pheromone were continually desorbed from the bodies of insects after mating was effected, males might be erroneously attracted to nonreceptive females or possibly to other males releasing pheromone from their body surfaces (Schneider, 1970).

What was the source of the esterase eluted in Ringer's solution from the cuticle of the antennae, legs, and wings? One possibility is the enzymes originated in the ducts or pore canals in the integument. Esterase(s) have been detected in the pore canals and in the endocuticle during moulting in larvae of *Calopodes ethlius* Stoll and are thought to be involved in the synthesis and maintenance of the epidermal wax layer or in some other cuticular process (Locke, 1959, 1974). Another possibility is that the esterases were leached from the subcuticular layers of the organs. Ahmad (1970) detected carboxyl-esterase activity histochemically in the subcuticular (epidermal) layer of the

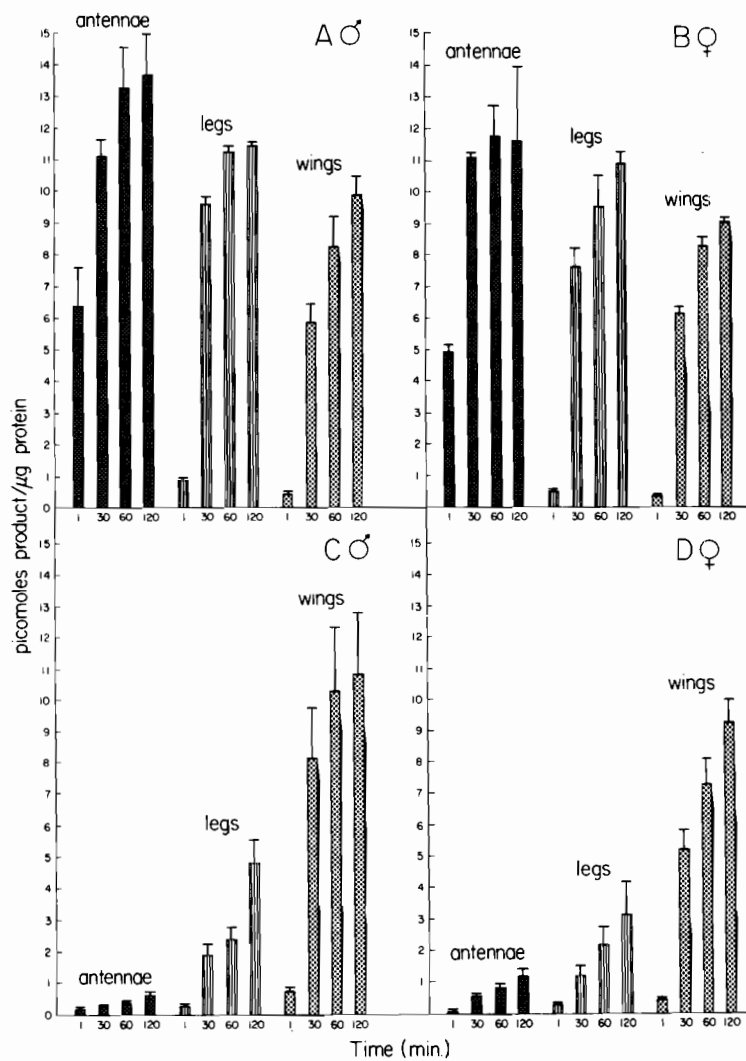


FIG. 1. Time courses of pheromone hydrolysis by esterases of *T. ni* male and female antennae, legs, and wings. (A and B) Homogenates as sources of pheromone-hydrolyzing esterase; (C and D) esterase eluted from the surfaces of antennae, legs, and wings into Ringer's solution for 12 hr. Each bar represents the standard deviation of the mean of four replications.

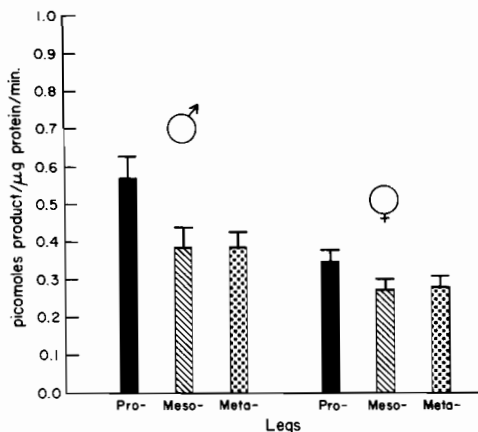


FIG. 2. Hydrolysis of pheromone by esterases obtained from homogenates of *T. ni* pro-, meso-, and metathoracic legs. Each bar represents the standard deviation of the mean of four replicates.

abdominal body wall of adult *Musca domestica* L. However, Kapin and Ahmad (1980) reported that in the integument of 5th instar gypsy moth larvae, the cuticle was the greater source of integumental esterase than the epidermis.

Although relatively low in esterase activity per unit of protein, the eluate of antennae contained considerably more total protein (6.4 and 3.4 μg protein/pair, male and female, respectively) than that of legs (1.4 and 2.6 μg protein/pair, male and female, respectively) or wings (2.2 and 3.2 μg protein/pair, male and female, respectively). If protein were released from the pore tubules in the olfactory sensilla, it would tend to dilute the cuticular esterase eluted from the epicuticle. This possibility becomes even more plausible when the reports of Riddiford (1970) and Seabrook (1977) are considered. Riddiford (1970) reported that a ^3H -labeled pheromone was bound by protein(s) in the antennae of saturniid moths, *Antheraea* spp., and that holding the organs in Ringer's solution for 30 min resulted in elution of the bound product. The proteins, which could be resynthesized by the antennae after the elution, were thought to originate in the pore tubule system of the olfactory sensilla. Whether the proteins were receptors or enzymes, however, was not clarified. Also, Seabrook (1977) indicated that the pore tubules in an olfactory sensillum of the eastern spruce budworm, *Choristoneura fumiferana* (Clemens), contained a proteinaceous fluid. Scanning electron micrographs of the olfactory trichodium of this insect showed that the sensillum was coated with a material that could be digested with protease. He concluded that the material removed was a protein that was extruded through the pores in the sensillum.

Considering the low specific activity of esterases eluted from the antennae of *T. ni*, the question arises as to how the insect removes the pheromone from the antennal surface. Although it has not been demonstrated that the cabbage looper moth cleans its antennae of pheromone during grooming behavior, many moths of Noctuidae do groom their antennae to keep them free of debris (Callahan and Carlisle, 1971). Thus, we considered the possibility that the prothoracic legs, which have a special antenna-grooming organ called a comb or epiphysis, have more esterase activity than the mesothoracic or metathoracic legs, which are not used in grooming. Figure 2 shows that the pheromone-degrading activity in the supernatant (20,000 g) from a homogenate of the prothoracic legs was higher than that of the mesothoracic or metathoracic legs. These data distinctly raise the possibility that the prothoracic legs are used to remove and degrade pheromone from the antennae during grooming. However, the data do not suggest the origin of the esterases—whether cuticular surface or interior tissue (i.e., muscle).

We noted that the specific activity of eluted esterases was higher for wings than for legs or antennae but are uncertain as to the significance of this finding. The females of *T. ni* have a relatively high nightly release rate of pheromone per individual (Bjostad et al., 1980). Possibly, therefore, the wings, because of their larger surface area, adsorb more pheromone during calling than other parts of the insect and require a more efficient means of degrading the pheromone.

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